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Staphylococcus aureus polynucleotides and sequences

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Abstract:

The present invention provides polynucleotide sequences of the genome of Staphylococcus aureus, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

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Claims:

1. Computer readable medium having recorded thereon a nucleotide sequence of the Staphylococcus aureus genome as depicted in SEQ ID NOS:1-5,191, a representative fragment thereof or a nucleotide sequence at least 95 % identical to a nucleotide sequence depicted in SEQ ID NOS:1-5,191.

2. Computer readable medium having recorded thereon any one of

the fragments of SEQ ID NOS:1-5,191 depicted in Tables 2 and 3 or a degenerate variant thereof.

3. The computer readable medium of claim 1, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.

4. The computer readable medium of claim 3, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.

5. A computer-based system for identifying fragments of the Staphylococcus aureus genome of commercial importance comprising the following elements: (a) a data storage means comprising the nucleotide sequence of SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95% identical to a nucleotide sequence of SEQ ID NOS:1-5,191; (b) search means for comparing a target sequence to the nucleotide sequence of the data storage means of step (a) to identify homologous sequence(s), and (c) retrieval means for obtaining said homologous sequence(s) of step (b).

6. A method for identifying commercially important nucleic acid fragments of the Staphylococcus aureus genome comprising the step of comparing a database comprising the nucleotide sequences depicted in SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95% identical to a nucleotide sequence of SEQ ID NOS:1-5,191 with a target sequence to obtain a nucleic acid molecule comprised of a complementary nucleotide sequence to said target sequence, wherein said target sequence is not randomly selected.

7. A method for identifying an expression modulating fragment of Staphylococcus aureus genome comprising the step of comparing a database comprising the nucleotide sequences depicted in SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOS:1-5,191 with a target sequence to obtain a nucleic acid molecule comprised of a complementary nucleotide sequence to said target sequence, wherein said target sequence comprises sequences known to regulate gene expression.

8. A protein-encoding nucleic acid fragment of the Staphylococcus aureus genome, wherein said fragment comprises the nucleotide sequence of any one of the fragments of SEQ ID NOS:1-5,191 depicted in Tables 2 and 3 or a nucleotide sequence at least 95% identical to such a nucleotide sequence, or a degenerate variant of any of the aforementioned sequences.

9. The nucleic acid fragment of claim 8 which is DNA.

10. The nucleic acid fragment of claim 8 which is RNA.

11. A vector comprising a fragment of claim 8.

12. A fragment of the Staphylococcus aureus genome, wherein said fragment modulates the expression of an operably liked open reading frame, wherein said fragment consists of the nucleotide sequence from about 10 to 200 bases in length which is 5' to any one of the open reading frames depicted in Tables 2 and 3 or a nucleotide sequence at least 95% identical to such a nucleotide sequence or a degenerate variant of any of the aforementioned sequences.

13. A vector comprising a fragment of claim 12.

14. A organism which has been altered to contain any one of the fragments of the Staphylococcus aureus genome of claim 8.

15. A method for producing a polypeptide in a host cell comprising the steps of: (a) incubating an organism of claim 14 under conditions where said fragment is expressed to produce said protein, and (b) isolating said protein.

16. An organism which has been altered to contain any one of the fragments of the Staphylococcus aureus genome of claim 12.

17. A method for regulating the expression of a nucleic acid molecule comprising the step of covalently attaching to said nucleic acid molecule a nucleic acid molecule consisting of the nucleotide sequence from about 30 to 300 bases 5' to any one of the fragments of the Staphylococcus aureus genome depicted in Seq ID Nos:1-5,191 and Tables 2 and 3 or a nucleotide sequence at least 95% identical to such a nucleotide sequence or a degenerate variant of any of the aforementioned sequences.

18. A nucleic acid molecule being a homolog of any of the fragments of the Staphylococcus aureus genome of SEQ ID NOS:1-5,191 and Tables 2 and 3, wherein said nucleic acid molecule is produced by a process comprising the steps of: (a) screening a genomic DNA library using as a probe a target sequence defined by any of SEQ ID NOS:1-5,191 and Tables 2 and 3, including fragments thereof; (b) identifying members of said library which contain sequences that hybridize to said target sequence; (c) isolating the nucleic acid molecules from said members identified in step (b).

19. A DNA molecule being a homolog of any one of the fragments of the Staphylococcus aureus genome of SEQ ID NOS:1-5,191 and Tables 2 and 3, wherein said nucleic acid molecule is produced by

a process comprising the steps of: (a) isolating mRNA, DNA, or cDNA produced from an organism; (b) amplifying nucleic acid molecules whose nucleotide sequence is homologous to amplification primers derived from said fragment of said Staphylococcus aureus genome to prime said amplification; (c) isolating said amplified sequences produced in step (b).

20. A polypeptide encoded by a fragment of claim 8.

21. An antibody which selectively binds to any one of the polypeptides of claim 20.

22. A kit for analyzing samples for the presence of polynucleotides derived from Staphylococcus aureus, comprising at least one polynucleotide containing a nucleotide sequence of any one of the fragments SEQ ID NOS:1-5,191 depicted in Tables 2 and 3 or a nucleotide sequence at least 95% identical thereto or a degenerate variant of any of the aforementioned sequences, that will hybridize to a staphylococcus aureus polynucleotide under stringent hybridization conditions, and a suitable container.

23. A Staphylococcus aureus polypeptide comprising an amino acid sequence identical to an amino acid sequence selected from the group consisting of SEQ ID NOS:5,192 to 5,255 or comprising an amino acid sequence having at least 95% identity to such a sequence.

24. A Staphylococcus aureus polypeptide antigen comprising at least one epitope derived from a Staphylococcus aureus polypeptide selected from the group consisting of SEQ ID NOS:5,192 to 5,255.

25. A polypeptide comprising at least one epitope encoded by a Staphylococcus aureus amino acid sequence selected from the group consisting of the epitopic sequences listed in Table 4.

26. The polypeptide of claim 24 or 26, wherein said polypeptide is fixed to a solid phase.

27. A diagnostic kit for detecting Staphylococcus aureus infection comprising (a) an isolated polypeptide antigen of claim 24, and (b) means for detecting the binding of an antibody contained in a biological fluid to said antigen.

28. A vaccine composition comprising a polypeptide of claim 24 present in a pharmaceutically acceptable carrier.

29. A method of vaccinating an individual against Staphylococcus aureus infection comprising, administering to an individual the vaccine composition of claim 28.

Description:

Conditions for incubating a DF, antigen or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the DF or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the Dfs, antigens or antibodies of the present invention. Examples of such assays can be found in Chard, T., An Introduction to Radioimmunoassay and Related Techniques, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.

R. et al., Techniques in Immunocytochemistry, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry; PCT publication WO95/32291, and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1985), all of which are hereby incorporated herein by reference.

The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises:(a) a first container comprising one of the Dfs, antigens or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following:wash reagents, reagents capable of detecting presence of a bound DF, antigen or antibody.

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion

from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody, antigen or DF.

Types of detection reagents include labelled nucleic acid probes, labelled secondary antibodies, or in the alternative, if the primary antibody is labelled, the enzymatic, or antibody binding reagents which are capable of reacting with the labelled antibody. One skilled in the art will readily recognize that the disclosed Dfs, antigens and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art. 4. Screening Assay for Binding Agents

Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents which bind to a protein encoded by one of the ORFs of the present invention or to one of the fragments and the Staphylococcus aureus fragment and contigs herein described.

In general, such methods comprise steps of: (a) contacting an agent with an isolated protein encoded by one of the ORFs of the present invention, or an isolated fragment of the Staphylococcus aureus genome; and (b) determining whether the agent binds to said protein or said fragment.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention.

Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby et al., Application of Synthetic Peptides: Antisense Peptides," In Synthetic Peptides, A User's Guide, W. H. Freeman, NY (1992), pp. 289-307, and Kaspczak et al., Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control.

One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251: 1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems.

Information contained in the sequences of the present invention can be used to design antisense and triple helix-forming oligonucleotides, and other DNA binding agents. 5. Pharmaceutical Compositions and Vaccines

The present invention further provides pharmaceutical agents which can be used to modulate the growth or pathogenicity of Staphylococcus aureus, or another related organism, in vivo or in vitro. As used herein, a "pharmaceutical agent" is defined as a composition of matter which can be formulated using known techniques to provide a pharmaceutical compositions. As used herein, the "pharmaceutical agents of the present invention" refers the pharmaceutical agents which are derived from the proteins encoded by the ORFs of the present invention or are agents which are identified using the herein described assays.

As used herein, a pharmaceutical agent is said to "modulate the growth or pathogenicity of Staphylococcus aureus or a related organism, in vivo or in vitro," when the agent reduces the rate of growth, rate of division, or viability of the organism in question. The pharmaceutical agents of the present invention can modulate the growth or pathogenicity of an organism in many fashions,

although an understanding of the underlying mechanism of action is not needed to practice the use of the pharmaceutical agents of the present invention. Some agents will modulate the growth or pathogenicity by binding to an important protein thus blocking the biological activity of the protein, while other agents may bind to a component of the outer surface of the organism blocking attachment or rendering the organism more prone to act the bodies nature immune system.

Alternatively, the agent may comprise a protein encoded by one of the ORFs of the present invention and serve as a vaccine. The development and use of vaccines derived from membrane associated polypeptides are well known in the art. The inventors have identified particularly preferred immunogenic Staphylococcus aureus polypeptides for use as vaccines. Such immunogenic polypeptides are described above and summarized in Table 4, below.

As used herein, a "related organism" is a broad term which refers to any organism whose growth or pathogenicity can be modulated by one of the pharmaceutical agents of the present invention. In general, such an organism will contain a homolog of the protein which is the target of the pharmaceutical agent or the protein used as a vaccine. As such, related organisms do not need to be bacterial but may be fungal or viral pathogens.

The pharmaceutical agents and compositions of the present invention may be administered in a convenient manner, such as by the oral, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, they are administered in an amount of at least about 1 mg/kg body weight and in most cases they will be administered in an amount not in excess of about 1 g/kg body weight per day. In most cases, the dosage is from about 0.1 mg/kg to about 10 g/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The agents of the present invention can be used in native form or can be modified to form a chemical derivative. As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed in, among other sources, REMINGTON'S PHARMACEUTICAL SCIENCES (1980) cited elsewhere herein.

For example, such moieties may change an immunological character of the functional derivative, such as affinity for a given antibody. Such changes in immunomodulation activity are measured by the appropriate assay, such as a competitive type immunoassay. Modifications of such protein properties as redox or thermal stability, biological half-life, hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers also may be effected in this way and can be assayed by methods well known to the skilled artisan.

The therapeutic effects of the agents of the present invention may be obtained by providing the agent to a patient by any suitable means (e.g., inhalation, intravenously, intramuscularly, subcutaneously, enterally, or parenterally). It is preferred to administer the agent of the present invention so as to achieve an effective concentration within the blood or tissue in which the growth of the organism is to be controlled. To achieve an effective blood concentration, the preferred method is to administer the agent by injection. The administration may be by continuous infusion, or by single or multiple injections.

In providing a patient with one of the agents of the present invention, the dosage of the administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, etc. In general, it is desirable to provide the recipient with a dosage of agent which is in the range of from about 1 pg/kg to 10 mg/kg (body weight of patient), although a lower or higher dosage may be administered. The therapeutically effective dose can be lowered by using combinations of the agents of the present invention or another agent.

As used herein, two or more compounds or agents are said to be administered "in combination" with each other when either (1) the physiological effects of each compound, or (2) the serum concentrations of each compound can be measured at the same time. The composition of the present invention can be administered concurrently with, prior to, or following the administration of the other agent.

The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to decrease the rate of growth (as defined above) of the target organism.

The administration of the agent(s) of the invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the agent(s) are provided in advance of any symptoms indicative of the organisms growth. The prophylactic administration of the agent(s) serves to prevent, attenuate, or decrease the rate of onset of any subsequent infection. When provided therapeutically, the agent(s) are provided at (or shortly

after) the onset of an indication of infection. The therapeutic administration of the compound(s) serves to attenuate the pathological symptoms of the infection and to increase the rate of recovery.

The agents of the present invention are administered to a subject, such as a mammal, or a patient, in a pharmaceutically acceptable form and in a therapeutically effective concentration. A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

The agents of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in REMINGTON'S PHARMACEUTICAL SCIENCES, 16 Ed., Osol, A., Ed., Mack Publishing, Easton PA (1980). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the agents of the present invention, together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Control release preparations may be achieved through the use of polymers to complex or absorb one or more of the agents of the present invention. The controlled delivery may be effectuated by a variety of well known techniques, including formulation with macromolecules such as, for example, polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate, adjusting the concentration of the macromolecules and the agent in the formulation, and by appropriate use of methods of incorporation, which can be manipulated to effectuate a desired time course of release.

Another possible method to control the duration of action by controlled release preparations is to incorporate agents of the present invention into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization with, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacylate) microcapsules, respectively, or in

colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in REMINGTON'S PHARMACEUTICAL SCIENCES (1980).

The invention further provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

In addition, the agents of the present invention may be employed in conjunction with other therapeutic compounds. 6. Shot-Gun Approach to Megabase DNA Sequencing

The present invention further demonstrates that a large sequence can be sequenced using a random shotgun approach. This procedure, described in detail in the examples that follow, has eliminated the up front cost of isolating and ordering overlapping or contiguous subclones prior to the start of the sequencing protocols.

Certain aspects of the present invention are described in greater detail in the examples that follow. The examples are provided by way of illustration. Other aspects and embodiments of the present invention are contemplated by the inventors, as will be clear to those of skill in the art from reading the present disclosure. ILLUSTRATIVE EXAMPLES LIBRARIES AND SEQUENCING 1. Shotgun Sequencing Probability Analysis

The overall strategy for a shotgun approach to whole genome sequencing follows from the Lander and Waterman (Landerman and Waterman, Genomics 2: 231 (1988)) application of the equation for the Poisson distribution. According to this treatment, the probability, P0, that any given base in a sequence of size L, in nucleotides, is not sequenced after a certain amount, n, in nucleotides, of random sequence has been determined can be calculated by the equation P0 = e <-m>, where m is L/n, the fold coverage." For instance, for a genome of 2.8 Mb, m=1 when 2.8 Mb of sequence has been randomly generated (1X coverage). At that point, P0 = e <-1> = 0.37.

The probability that any given base has not been sequenced is the same as the probability that any region of the whole sequence L has not been determined and, therefore, is equivilent to the fraction of the whole sequence that has yet to be determined. Thus, at one-fold coverage, approximately 37% of a polynucleotide of size L, in nucleotides has not been sequenced. When 14 Mb of sequence has

been generated, coverage is 5X for a .2.8 Mb and the unsequenced fraction drops to .0067 or 0.67%. 5X coverage of a 2.8 Mb sequence can be attained by sequencing approximately 17,000 random clones from both insert ends with an average sequence read length of 410 bp.

Similarly, the total gap length, G, is determined by the equation G = Le <-m>, and the average gap size, g, follows the equation, g = L/n. Thus, 5X coverage leaves about 240 gaps averaging about 82 bp in size in a sequence of a polynucleotide 2.8 Mb long.

The treatment above is essentially that of Lander and Waterman, Genomics 2: 231 (1988). 2. Random Library Construction

In order to approximate the random model described above during actual sequencing, a nearly ideal library of cloned genomic fragments is required. The following library construction procedure was developed to achieve this end.

Staphylococcus aureus DNA was prepared by phenol extraction. A mixture containing 600 ug DNA in 3.3 ml of 300 mM sodium acetate, 10 mM Tris-HCl, 1 mM Na-EDTA, 30% glycerol was sonicated for 1 min. at 0 DEG C in a Branson Model 450 Sonicator at the lowest energy setting using a 3 mm probe. The sonicated DNA was ethanol precipitated and redissolved in 500 ul TE buffer.

To create blunt-ends, a 100 ul aliquot of the resuspended DNA was digested with 5 units of BAL31 nuclease (New England BioLabs) for 10 min at 30 DEG C in 200 ul BAL31 buffer . The digested DNA was phenol-extracted, ethanol-precipitated, redissolved in 100 ul TE buffer, and then size-fractionated by electrophoresis through a 1.0% low melting temperature agarose gel. The section containing DNA fragments 1.6-2.0 kb in size was excised from the gel, and the LGT agarose was melted and the resulting solution was extracted with phenol to separate the agarose from the DNA. DNA was ethanol precipitated and redissolved in 20 ul of TE buffer for ligation to vector.

A two-step ligation procedure was used to produce a plasmid library with 97% inserts, of which >99% were single inserts. The first ligation mixture (50 ul) contained 2 ug of DNA fragments, 2 ug pUC18 DNA (Pharmacia) cut with SmaI and dephosphorylated with bacterial alkaline phosphatase, and 10 units of T4 ligase (GIBCO/BRL) and was incubated at 14 DEG C for 4 hr. The ligation mixture then was phenol extracted and ethanol precipitated, and the precipitated DNA was dissolved in 20 ul TE buffer and electrophoresed on a 1.0% low melting agarose gel. Discrete bands in a ladder were visualized by ethidium bromide-staining and UV illumination and identified by size as insert (i), vector (v), v+i, v+2i, v+3i, etc. The portion of the gel containing v+i DNA was excised and the v+i DNA was recovered and

resuspended into 20 ul TE.

The v+i DNA then was blunt-ended by T4 polymerase treatment for 5 min. at 37 DEG C in a reaction mixture (50 ul) containing the v+i linears, 500 uM each of the 4 dNTPs, and 9 units of T4 polymerase (New England BioLabs), under recommended buffer conditions. After phenol extraction and ethanol precipitation the repaired v+i linears were dissolved in 20 ul TE. The final ligation to produce circles was carried out in a 50 ul reaction containing 5 ul of v+i linears and 5 units of T4 ligase at 14 DEG C overnight. After 10 min. at 70 DEG C the following day, the reaction mixture was stored at -20 DEG C.

This two-stage procedure resulted in a molecularly random collection of single-insert plasmid recombinants with minimal contamination from double-insert chimeras (<1%) or free vector (<3%).

Since deviation from randomness can arise from propagation the DNA in the host, E.coli host cells deficient in all recombination and restriction functions (A. Greener, Strategies 3 (1):5 (1990)) were used to prevent rearrangements, deletions, and loss of clones by restriction. Furthermore, transformed cells were plated directly on antibiotic diffusion plates to avoid the usual broth recovery phase which allows multiplication and selection of the most rapidly growing cells.

Plating was carried out as follows. A 100 ul aliquot of Epicurian Coli SURE II Supercompetent Cells (Stratagene 200152) was thawed on ice and transferred to a chilled Falcon 2059 tube on ice. A 1.7 ul aliquot of 1.42 M beta-mercaptoethanol was added to the aliquot of cells to a final concentration of 25 mM. Cells were incubated on ice for 10 min. A 1 ul aliquot of the final ligation was added to the cells and incubated on ice for 30 min. The cells were heat pulsed for 30 sec. at 42 DEG C and placed back on ice for 2 min. The outgrowth period in liquid culture was eliminated from this protocol in order to minimize the preferential growth of any given transformed cell.

Instead the transformation mixture was plated directly on a nutrient rich SOB plate containing a 5 ml bottom layer of SOB agar (5% SOB agar: 20 g tryptone, 5 g yeast extract, 0.5 g NaCl, 1.5% Difco Agar per liter of media). The 5 ml bottom layer is supplemented with 0.4 ml of 50 mg/ml ampicillin per 100 ml SOB agar. The 15 ml top layer of SOB agar is supplemented with 1 ml X-Gal (2%), 1 ml MgCl2 (1 M), and 1 ml MgSO4/100 ml SOB agar. The 15 ml top layer was poured just prior to plating. Our titer was approximately 100 colonies/10 ul aliquot of transformation.

All colonies were picked for template preparation regardless of size. Thus, only clones lost due to "poison" DNA or deleterious

gene products would be deleted from the library, resulting in a slight increase in gap number over that expected. 3. Random DNA Sequencing

High quality double stranded DNA plasmid templates were prepared using an alkaline lysis method developed in collaboration with 5Prime -> 3Prime Inc. (Boulder, CO). Plasmid preparation was performed in a 96-well format for all stages of DNA preparation from bacterial growth through final DNA purification. Average template concentration was determined by running 25% of the samples on an agarose gel. DNA concentrations were not adjusted.

Templates were also prepared from a Staphylococcus aureus lambda genomic library. An unamplified library was constructed in Lambda DASH II vector (Stratagene). Staphylococcus aureus DNA (> 100 kb) was partially digested in a reaction mixture (200 ul) containing 50 ug DNA, 1X Sau3AI buffer, 20 units Sau3AI for 6 min. at 23 C. The digested DNA was phenol-extracted and centrifuges over a 10- 40% sucroce gradient. Fractions containing genomic DNA of 15-25 kb were recovered by precipitation . One ul of fragments was used with 1 ul of DASHII vector (Stratagene) in the recommended ligation reaction. One ul of the ligation mixture was used per packaging reaction following the recommended protocol with the Gigapack II XL Packaging Extract Phage were plated directly without amplification from the packaging mixture (after dilution with 500 ul of recommended SM buffer and chloroform treatment).

Yield was about 2.5x10 <9> pfu/ul.

An amplified library was prepared from the primary packaging mixture according to the manufactureer's protocol. The amplified library is stored frozen in 7% dimethylsulfoxide. The phage titer is approximately 1x10 <9> pfu/ml.

Mini-liquid lysates (0.1ul) are prepared from randomly selected plaques and template is prepared by long range PCR. Samples are PCR amplified using modified T3 and T7 primers, and Elongase Supermix (LTI).

Sequencing reactions are carried out on plasmid templates using a combination of two workstations (BIOMEK 1000 and Hamilton Microlab 2200) and the Perkin-Elmer 9600 thermocycler with Applied Biosystems PRISM Ready Reaction Dye Primer Cycle Sequencing Kits for the M13 forward (M13-21) and the M13 reverse (M13RP1) primers. Dye terminator sequencing reactions are carried out on the lambda templates on a Perkin-Elmer 9600 Thermocycler using the Applied Biosystems Ready Reaction Dye Terminator Cycle Sequencing kits. Modified T7 and T3 primers are used to sequence the ends of the inserts from the Lambda

DASH II library. Sequencing reactions are on a combination of AB 373 DNA Sequencers and ABI 377 DNA sequencers. All of the dye terminator sequencing reactions are analyzed using the 2X 9 hour module on the AB 377.

Dye primer reactions are analyzed on a combination of ABI 373 and ABI 377 DNA sequencers. The overall sequencing success rate very approximately is about 85% for M13-21 and M13RP1 sequences and 65% for dye-terminator reactions. The average usable read length is 485 bp for M13-21 sequences, 445bp for M13RP1 sequences, and 375 bp for dye-terminator reactions. 4. Protocol for Automated Cycle Sequencing

The sequencing was carried out using Hamilton Microstation 2200, Perkin Elmer 9600 thermocyclers, ABI 373 and ABI 377 Automated DNA Sequencers. The Hamilton combines pre-aliquoted templates and reaction mixes consisting of deoxy- and dideoxynucleotides, the thermostable Taq DNA polymerase, fluorescently-labelled sequencing primers, and reaction buffer. Reaction mixes and templates were combined in the wells of a 96-well thermocycling plate and transferred to the Perkin Elmer 9600 thermocycler. Thirty consecutive cycles of linear amplification (i.e.., one primer synthesis) steps were performed including denaturation, annealing of primer and template, and extension; i.e., DNA synthesis. A heated lid with rubber gaskets on the thermocycling plate prevents evaporation without the need for an oil overlay.

Two sequencing protocols were used: one for dye-labelled primers and a second for dye-labelled dideoxy chain terminators. The shotgun sequencing involves use of four dye-labelled sequencing primers, one for each of the four terminator nucleotide. Each dye-primer was labelled with a different fluorescent dye, permitting the four individual reactions to be combined into one lane of the 373 or 377 DNA Sequencer for electrophoresis, detection, and base-calling. ABI currently supplies premixed reaction mixes in bulk packages containing all the necessary non-template reagents for sequencing. Sequencing can be done with both plasmid and PCR-generated templates with both dye-primers and dye- terminators with approximately equal fidelity, although plasmid templates generally give longer usable sequences.

Thirty-two reactions were loaded per ABI 373 Sequencer each day and 96 samples can be loaded on an ABI 377 per day. Electrophoresis was run overnight (ABI 373) or for 2 1/2 hours (ABI 377) following the manufacturer's protocols. Following electrophoresis and fluorescence detection, the ABI 373 or ABI 377 performs automatic lane tracking and base-calling. The lane-tracking was confirmed visually. Each sequence electropherogram (or fluorescence lane trace) was inspected visually and assessed for quality. Trailing sequences of low quality were removed and the

sequence itself was loaded via software to a Sybase database (archived daily to 8mm tape). Leading vector polylinker sequence was removed automatically by a software program.

Average edited lengths of sequences from the standard ABI 373 or ABI 377 were around 400 bp and depend mostly on the quality of the template used for the sequencing reaction. INFORMATICS 1. Data Management

A number of information management systems for a large-scale sequencing lab have been developed. (For review see, for instance, Kerlavage et al., Proceedings of the Twenty-Sixth Annual Hawaii International Conference on System Sciences, IEEE Computer Society Press, Washington D. C., 585 (1993)) The system used to collect and assemble the sequence data was developed using the Sybase relational database management system and was designed to automate data flow whereever possible and to reduce user error. The database stores and correlates all information collected during the entire operation from template preparation to final analysis of the genome.

Because the raw output of the ABI 373 Sequencers was based on a Macintosh platform and the data management system chosen was based on a Unix platform, it was necessary to design and implement a variety of multi- user, client-server applications which allow the raw data as well as analysis results to flow seamlessly into the database with a minimum of user effort. 2. Assembly

An assembly engine (TIGR Assembler) developed for the rapid and accurate assembly of thousands of sequence fragments was enployed to generate contigs. The TIGR assembler simultaneously clusters and assembles fragments of the genome. In order to obtain the speed necessary to assemble more than 10 <4> fragments, the algorithm builds a hash table of 12 bp oligonucleotide subsequences to generate a list of potential sequence fragment overlaps. The number of potential overlaps for each fragment determines which fragments are likely to fall into repetitive elements. Beginning with a single seed sequence fragment, TIGR Assembler extends the current contig by attempting to add the best matching fragment based on oligonucleotide content.

The contig and candidate fragment are aligned using a modified version of the Smith-Waterman algorithm which provides for optimal gapped alignments (Waterman, M. S., Methods in Enzymology 164: 765 (1988)). The contig is extended by the fragment only if strict criteria for the quality of the match are met. The match criteria include the minimum length of overlap, the maximum length of an unmatched end, and the minimum percentage match. These criteria are automatically lowered by the algorithm in regions of minimal coverage and raised in regions with a possible repetitive element. The number of potential

overlaps for each fragment determines which fragments are likely to fall into repetitive elements.

Fragments representing the boundaries of repetitive elements and potentially chimeric fragments are often rejected based on partial mismatches at the ends of alignments and excluded from the current contig. TIGR Assembler is designed to take advantage of clone size information coupled with sequencing from both ends of each template. It enforces the constraint that sequence fragments from two ends of the same template point toward one another in the contig and are located within a certain ranged of base pairs (definable for each clone based on the known clone size range for a given library). 3. Identifying Genes

The predicted coding regions of the Staphylococcus aureus genome were initially defined with the program zorf, which finds ORFs of a minimum length. The predicted coding region sequences were used in searches against a database of all Staphylococcus aureus nucleotide sequences from GenBank (release 92.0), using the BLASTN search method to identify overlaps of 50 or more nucleotides with at least a 95% identity. Those ORFs with nucleotide sequence matches are shown in Table 1. The ORFs without such matches were translated to protein sequences and and compared to a non-redundant database of known proteins generated by combining the Swiss-prot, PIR and GenPept databases. ORFs of at least 80 amino acids that matched a database protein with BLASTP probability less than or equal to 0.01 are shown in Table 2. The table also lists assigned functions based on the closest match in the databases.

ORFs of at least 120 amino acids that did not match protein or nucleotide sequences in the databases at these levels are shown in Table 3. ILLUSTRATIVE APPLICATIONS 1. Production of an Antibody to a Staphylococcus aureus Protein

Substantially pure protein or polypeptide is isolated from the transfected or transformed cells using any one of the methods known in the art. The protein can also be produced in a recombinant prokaryotic expression system, such as E. coli, or can by chemically synthesized. Concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows. 2. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., Nature 256:495 (1975) or modifications of the methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms

of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued.

Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., Meth. Enzymol. 70:419 (1980), and modified methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, New York. Section 21-2 (1989). 3. Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than other and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigenadministered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al., J. Clin. Endocrinol. Metab. 33:988-991 (1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. et al., Chap. 19 in:Handbook of Experimental Immunology, Wier, D., ed, Blackwell (1973). Plateau concentration of antibody is usually in the range of 0. 1 to 0. 2 mg/ml of serum (about 12M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in:Manual of Clinical Immunology, second edition, Rose and Friedman, eds., Amer. Soc. For Microbiology, Washington, D. C. (1980)

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi- quantitatively or qualitatively to identify

the presence of antigen in a biological sample. In addition, they are useful in various animal models of Staphylococcal disease known to those of skill in the art as a means of evaluating the protein used to make the antibody as a potential vaccine target or as a means of evaluating the antibody as a potential immunothereapeutic reagent. 3. Preparation of PCR Primers and Amplification of DNA

Various fragments of the Staphylococcus aureus genome, such as those of Tables 1-3 and SEQ ID NOS:1-5,191 can be used, in accordance with the present invention, to prepare PCR primers for a variety of uses. The PCR primers are preferably at least 15 bases, and more preferably at least 18 bases in length. When selecting a primer sequence, it is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. The PCR primers and amplified DNA of this Example find use in the Examples that follow. 4. Gene expression from DNA Sequences Corresponding to ORFs

A fragment of the Staphylococcus aureus genome provided in Tables 1-3 is introduced into an expression vector using conventional technology. Techniques to transfer cloned sequences into expression vectors that direct protein translation in mammalian, yeast, insect or bacterial expression systems are well known in the art. Commercially available vectors and expression systems are available from a variety of suppliers including Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence may be optimized for the particular expression organism, as explained by Hatfield et al., U. S. Patent No. 5,082,767, incorporated herein by this reference.

The following is provided as one exemplary method to generate polypeptide(s) from cloned ORFs of the Staphylococcus aureus genome fragment. Bacterial ORFs generally lack a poly A addition signal. The addition signal sequence can be added to the construct by, for example, splicing out the poly A addition sequence from pSG5 (Stratagene) using BglI and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene) for use in eukaryotic expression systems. pXT1 contains the LTRs and a portion of the gag gene of Moloney Murine Leukemia Virus. The positions of theLTRs in the construct allow efficient stable transfection. The vector includes the Herpes Simplex thymidine kinase promoter and the selectable neomycin gene.

The Staphylococcus aureus DNA is obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the Staphylococcus aureus DNA and containing restriction endonuclease sequences for PstI incorporated into the 5' primer and BglII at the 5' end of the corresponding Staphylococcus aureus

DNA 3' primer, taking care to ensure that the Staphylococcus aureus DNA is positioned such that its followed with the poly A addition sequence. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with BglII, purified and ligated to pXT1, now containing a poly A addition sequence and digested BglII.

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 ug/ml G418 (Sigma, St. Louis, Missouri). The protein is preferably released into the supernatant. However if the protein has membrane binding domains, the protein may additionally be retained within the cell or expression may be restricted to the cell surface. Since it may be necessary to purify and locate the transfected product, synthetic 15-mer peptides synthesized from the predicted Staphylococcus aureus DNA sequence are injected into mice to generate antibody to the polypeptide encoded by the Staphylococcus aureus DNA.

Alternativly and if antibody production is not possible, the Staphylococcus aureus DNA sequence is additionally incorporated into eukaryotic expression vectors and expressed as, for example, a globin fusion. Antibody to the globin moiety then is used to purify the chimeric protein. Corresponding protease cleavage sites are engineered between the globin moiety and the polypeptide encoded by the Staphylococcus aureus DNA so that the latter may be freed from the formed by simple protease digestion. One useful expression vector for generating globin chimerics is pSG5 (Stratagene). This vector encodes a rabbit globin. Intron II of the rabbit globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques are well known to those skilled in the art of molecular biology.

Standard methods are published in methods texts such as Davis et al., cited elsewhere herein, and many of the methods are available from the technical assistance representatives from Stratagene, Life Technologies, Inc., or Promega. Polypeptides of the invention also may be produced using in vitro translation systems such as in vitro ExpressTM Translation Kit (Stratagene).

While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention.

All patents, patent applications and publications referred to above are hereby incorporated by reference. EMI62.1 EMI63.1 EMI64.1 EMI65.1 EMI66.1 EMI67.1 EMI68.1 EMI69.1 EMI70.1 EMI71.1

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